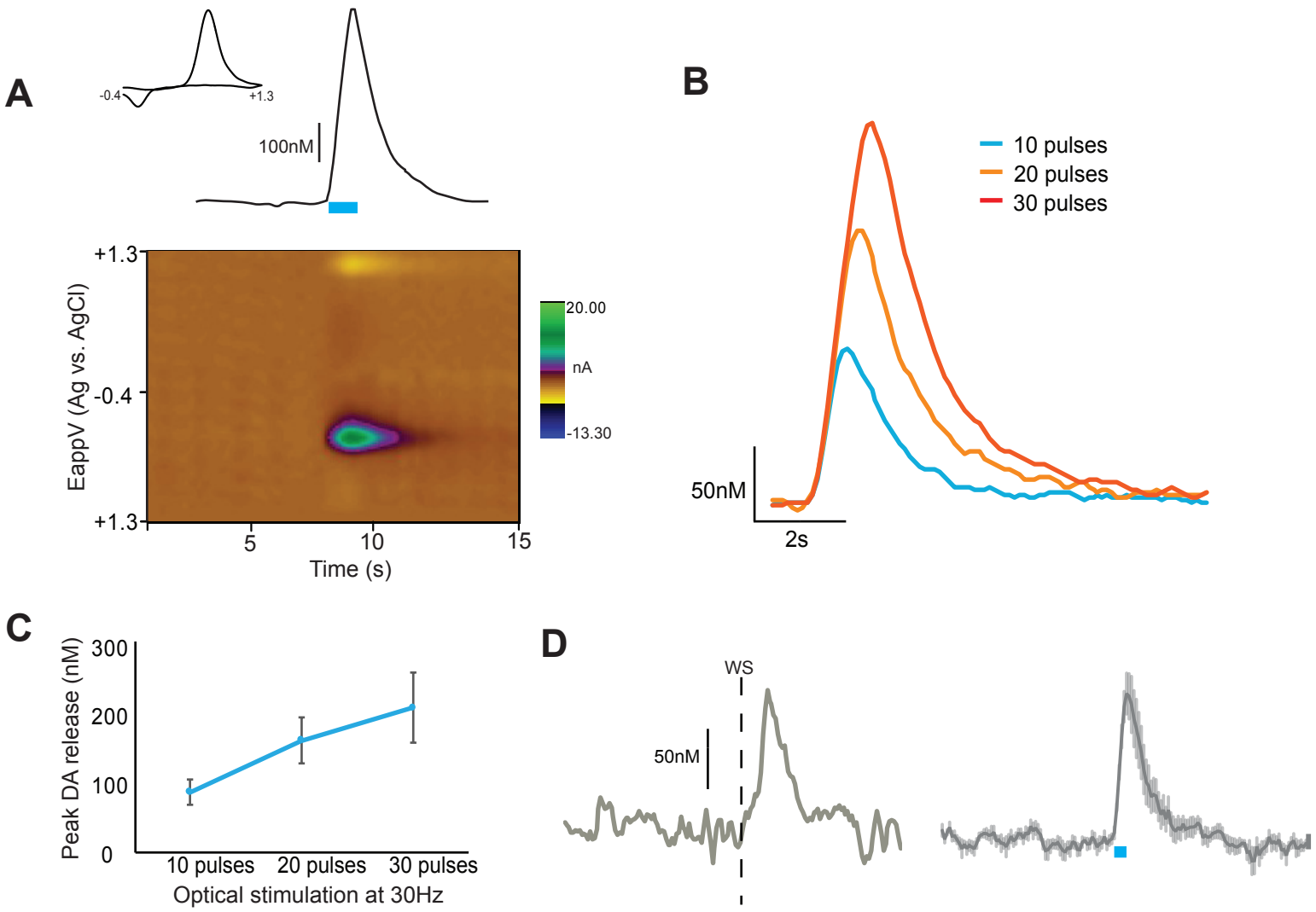
A
-0.4
+1.3
100nM
+1.3
-0.4
+1.3
EappV (Ag vs. AgCl)
5
10
15
Time (s)
20.00
nA
-13.30
B
10 pulses
20 pulses
30 pulses
50nM
2s
C
300
200
100
0
Peak DA release (nM)
10 pulses
20 pulses
30 pulses
Optical stimulation at 30Hz
D
WS
50nM

Figure S1

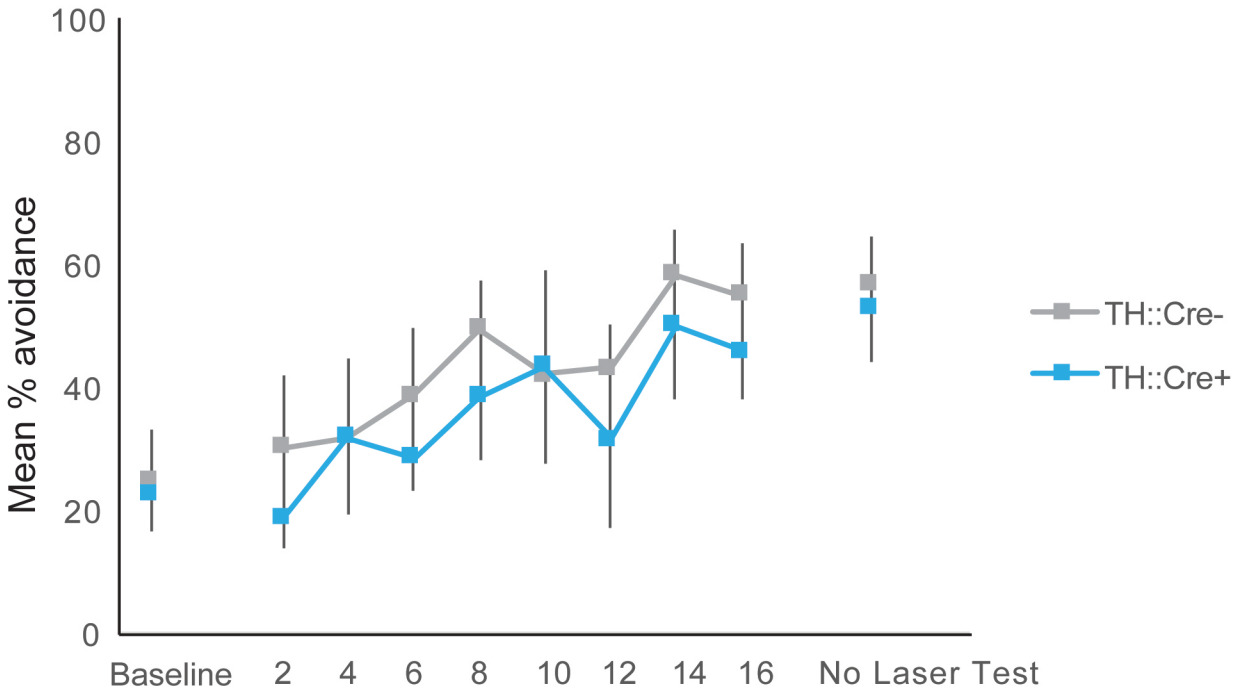
100
80
60
40
20
0
Mean % avoidance
Baseline
2
4
6
8
10
12
14
16
No Laser Test
TH::Cre-
TH::Cre+

Figure S2

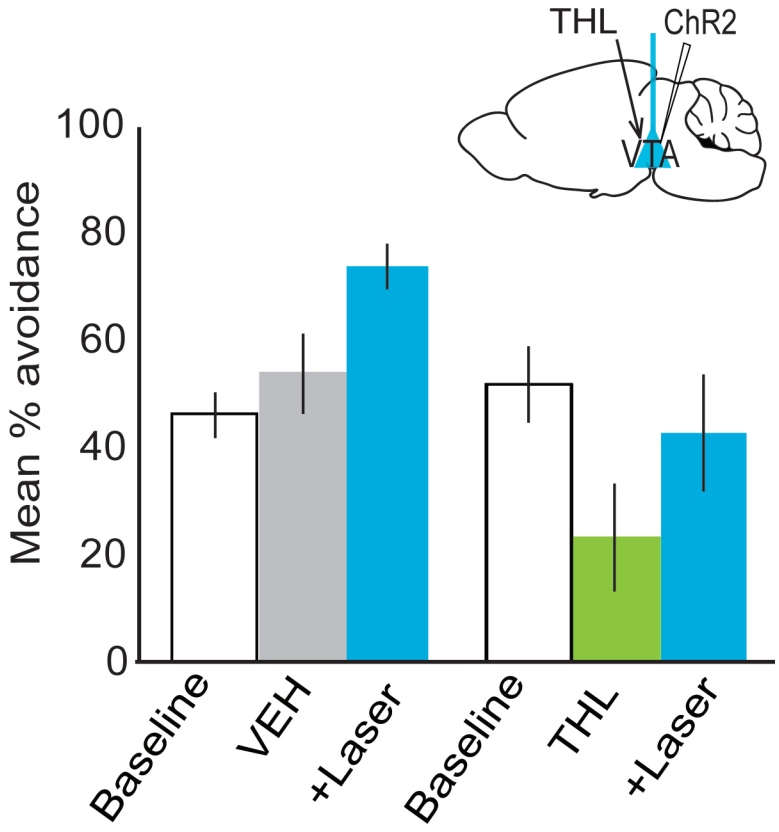
THL
ChR2
VTA
Mean % avoidance
100
80
60
40
20
0
Baseline
VEH
+Laser
Baseline
THL
+Laser

Figure S3

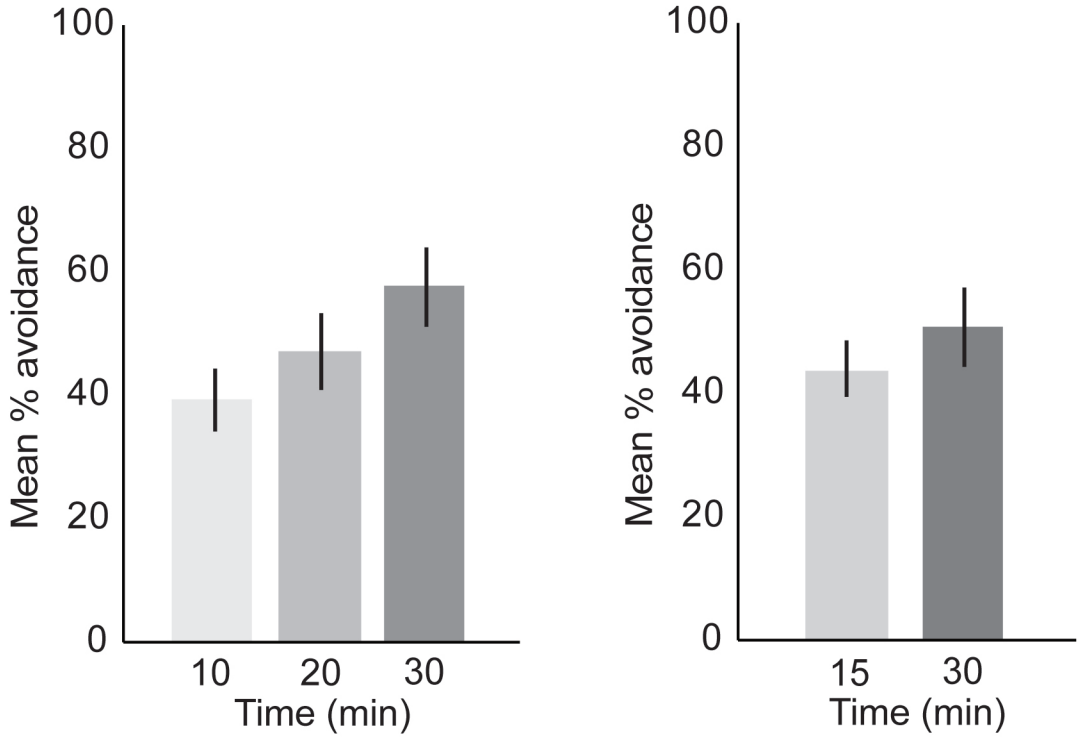
100
80
60
40
20
0
Mean % avoidance
10
20
30
Time (min)
100
80
60
40
20
0
Mean % avoidance
15
30
Time (min)

Figure S4

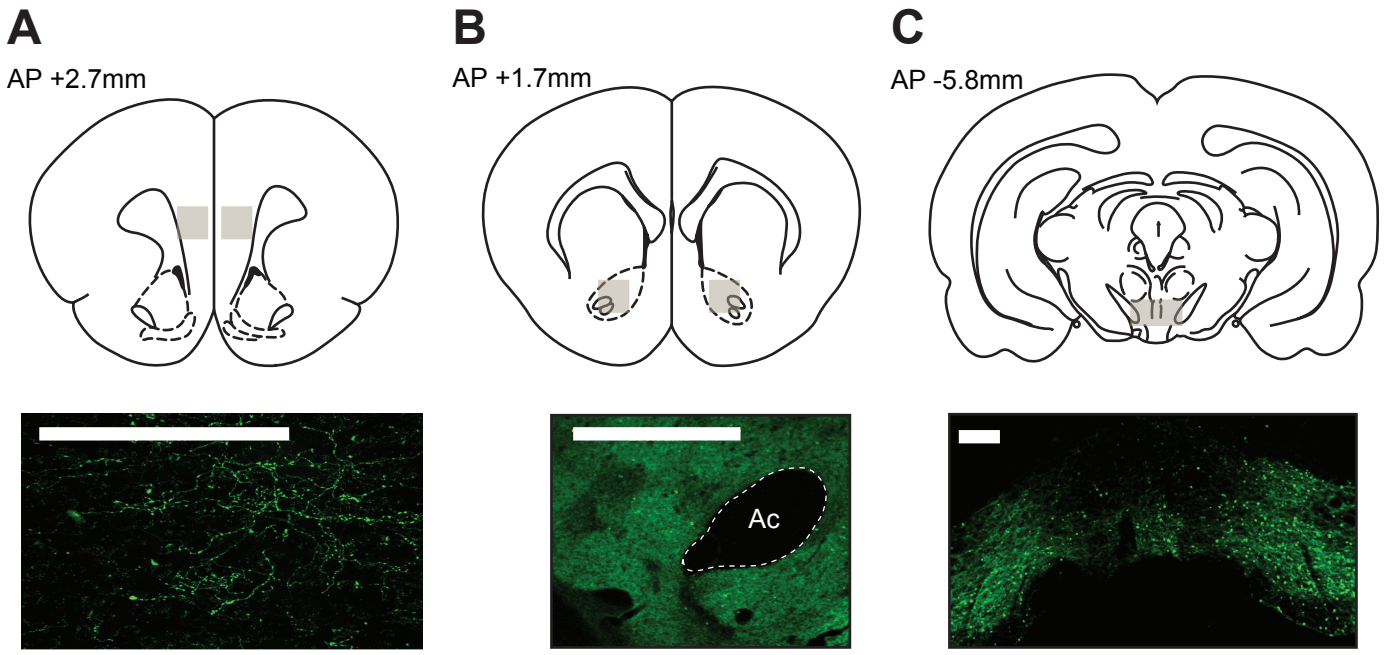

Figure S5

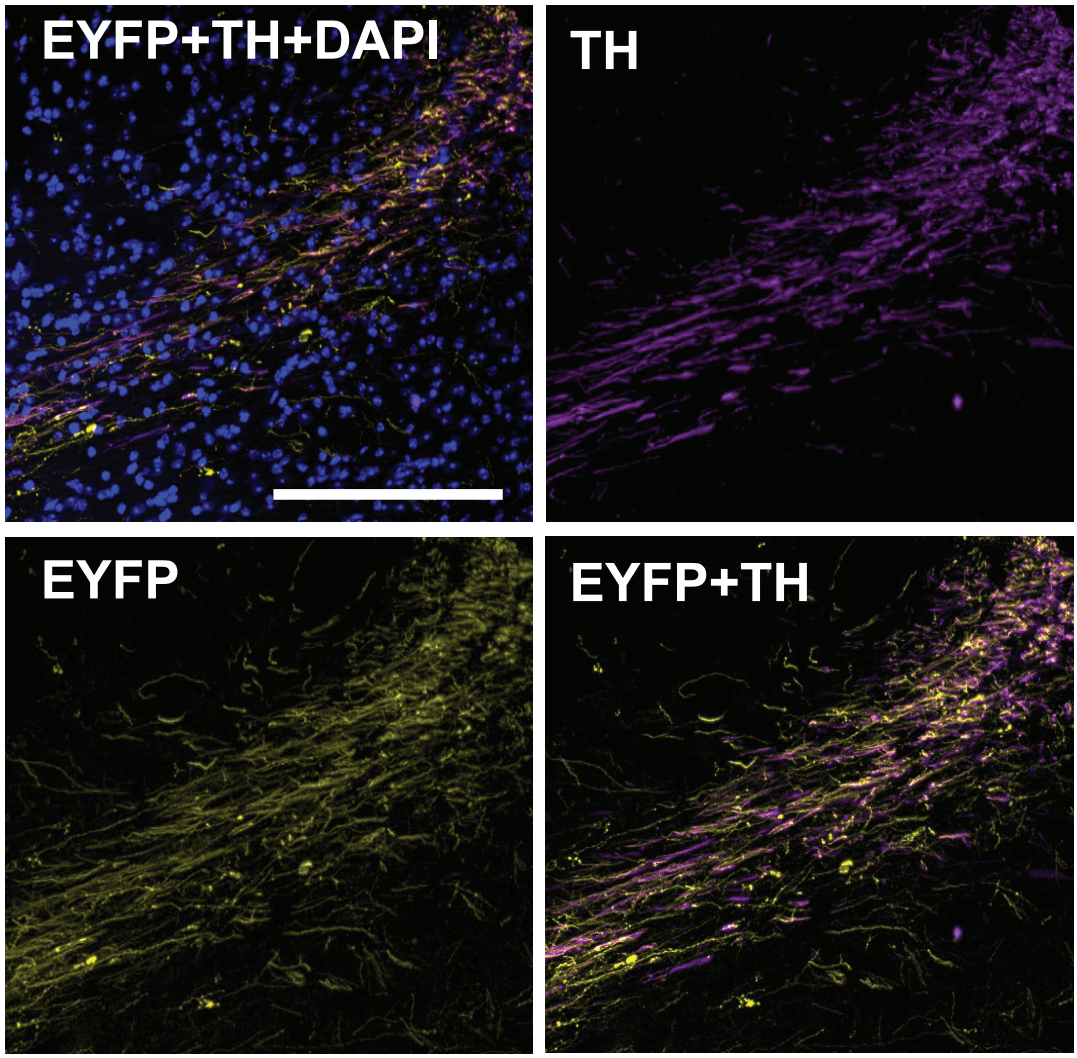

Figure S6

Figure S1. Optogenetic stimulation of VTA dopamine cells results in NAcC DA release in TH::Cre+ rats, see also Figure 1. (**A)** FSCV color plot shows the voltammetric current (encoded in color) plotted against the applied potential (y-axis) and the acquisition time (x-axis). Following application of a single optical stimulation train of 60 pulses delivered at 60 Hz (blue line above color plot), NAcc dopamine release rapidly increased. Corresponding DA concentration over time is plotted above (left) along with cyclic voltammogram (left). **(B)** Representative example pulse-response plot of optically-induced NAcC dopamine release following 30, 60, or 90 pulses at 30Hz. **(C)** Average peak DA release (nM; y-axis) following application of a single optical pulse train consisting of 10, 20, or 30 pulses delivered at 30Hz. **(D)** Comparison of a representative NAcC DA transient to the WS prior to avoidance (left) and average optically-induced DA release to a 10 pulse at 20Hz stimulation train (error bars represent SEM).

Figure S2. Optogenetic stimulation of VTA DA from the start of training does not affect the rate of avoidance learning, see also Figure 5. (A) Mean percent avoidance in TH::Cre+ and TH::Cre- rats transduced with ChR2 and implanted with bilateral optical fibers in the VTA during a single initial no-stimulation 30min “Baseline” avoidance session, and during each subsequent session during which all animals received laser stimulation (10 pulses at 20Hz at the presentation of each WS. Data are averaged into two-session bins to reduce behavioral variability. The day after the 16th stimulation session, all animals received a single 30min no-stimulation session to determine if avoidance behavior would be sustained without laser facilitation of VTA DA activation. There were no differences in baseline performance between groups, nor was the any difference in

performance as animals learned the avoidance task. On the final “No Laser Test”, all animals maintained similar avoidance responding as observed in the presence of laser stimulation.

Figure S3. Antagonism of 2-AG synthesis attenuates avoidance, see also Figure 5. Mean percent avoidance in TH::Cre+ rats transduced with ChR2 and receiving optical stimulation in the VTA following infusion of either VEH or THL into the VTA (0.5 μg/side).

Figure S4. Avoidance responding does not change over the course of a baseline session, see also STAR Methods. (A) Mean percent avoidance over the course of a 30min baseline session. Data has been averaged into three successive 10min bins to show no significant change in avoidance responding over the session. **(B)** The identical behavioral data, now averaged into two 15min bins to show no significant change in avoidance as the session progressed. These analyses justify shortening our sessions from one hour (as in Figure 1B) to 30min in subsequent experiments and to average avoidance counts over 10 or 15min bins following stimulation or pharmacological manipulations.

Figure S5. Histological confirmation of cannulae, fiber and electrode placement and virus expression, see also STAR Methods. Representative plates from the rat brain atlas [S1] with AP denoted from Bregma (top) and EYFP fluorescence confirming ChR2 expression (bottom), in the PFC **(A)**, NAcC **(B)** and VTA **(C)**. Gray boxes indicate region in which all included animals’ optical fibers, voltammetric electrodes, and/or

infusion cannulae terminated. Scale bar represents 200μm. “Ac” denotes position of the anterior commissure.

Figure S6. Immunohistochemical confirmation of ChR2-EYFP expression in TH+ processes in the NAcC, see also STAR Methods. Top left panel shows ChR2-EYFP (histological amplification using GFP antibody) expression (yellow) co-localized with tyrosine hydroxylase (Cy5, purple), and a lack of co-localized DAPI (blue), indicating expression is only present in DA terminals in NAcC, and not cell bodies. Scale bar represents 100μm. Top right panel shows anti-TH staining via Cy5 alone. Bottom left panel E shows EYFP, which is co-expressed with ChR2. Bottom right panel shows co-localization of TH and EYFP alone for clarity.

Supplemental References

S1. Paxinos, G., & Watson, C. (2005). *The rat brain in stereotaxic coordinates: Elsevier Academic Press*.